

Aus der II. Medizinischen Abteilung des Bürgerspitals Basel

Vorsteher: Prof. Dr. H. Ludwig

Arbeit unter Leitung von Dr. A. Walser

Influence of ACTH on PBI with Respect to Diurnal Variation

Inaugural-Dissertation

zur Erlangung der Doktorwürde der Medizinischen Fakultät
der Universität Basel

vorgelegt von

Arnold A. Heiger,

von Brooklyn, N.Y., USA

Basel 1959

Von der Medizinischen Fakultät
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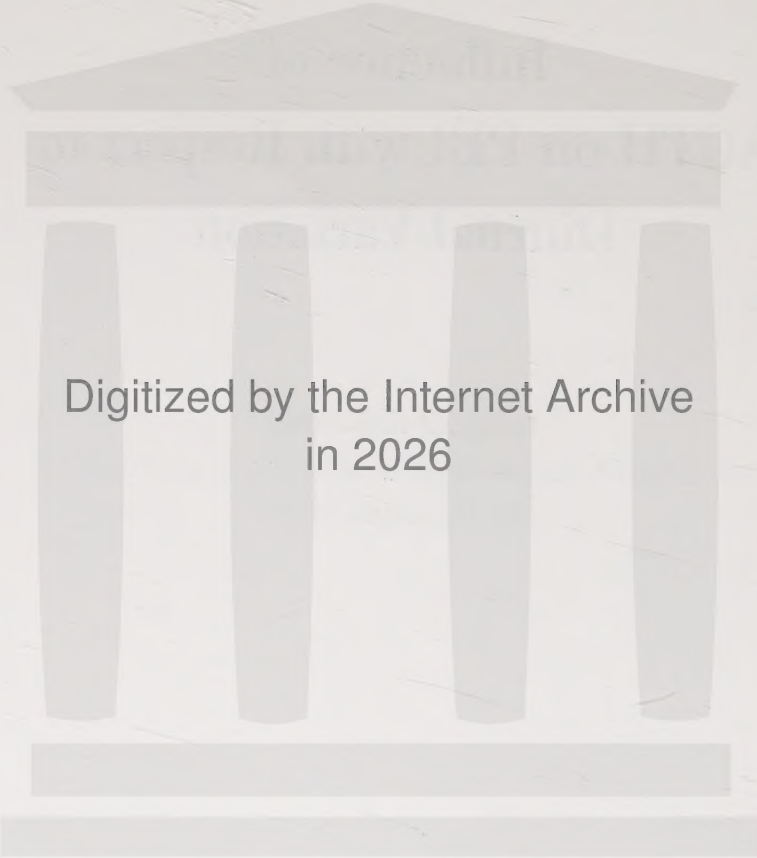
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The problem of the interrelationship between the adrenal gland and the thyroid gland has been studied by many authors and an interrelationship does seem to exist, but there is, as yet, no definitive proof of the method or mechanism of this relationship. Cortisone and Adrenocortical Tropic Hormone (hereafter to be referred to as ACTH) have been shown, by most authors, to depress thyroid function when given as prolonged therapy. The object of this paper is to show that exogenous ACTH will decrease the circulating Protein Bound Iodine during its administration and that this depressive action is variable as to length of time. The problem of the existence of a diurnal fluctuation in the Protein Bound Iodine levels will also be considered.

One of the most commonly employed laboratory tests to evaluate thyroid function is that of measuring the Protein Bound Iodine content of the blood serum. This Protein Bound Iodine (PBI) is a measure of the circulating iodine which is bound to the protein molecule¹. It provides a direct quantitative estimation of the level of circulating thyroid hormone². It has been shown that the level of PBI is affected by certain drugs³ but that it is relatively constant as concerns age⁴, sex⁵, activity⁶, and conditions of health⁷ including extrathyroidal causes of hypermetabolism¹. *Gottschalk*⁸ showed a slight depression of PBI values with increasingly colder weather in those subjects removed to northern lands and a higher PBI value in Eskimos than in inhabitants of the temperate zones. Females show cyclic variations in the PBI level⁹ with a midcyclic rise and a decrease during the postovulatory phase. *Heinmann*¹⁰ has found an elevation in the PBI as early as the third week of pregnancy with a fall to normal after delivery.

*Basset*¹² showed that the bulk of the PBI is associated with the "crude albumin fractions precipitated below a molarity of 3.0 of ammonium sulfate. Those proteins which were soluble in this concentration of ammonium sulfate did not contain an appreciable amount of PBI. *McClendon*¹³ reported that the PBI in the erythrocytes themselves is almost as high as that found in plasma although *Goodman and Gillman*¹⁴ state that little or none of the organic iodine in the circulation can be detected in the red blood cells. It is well known that certain medications which contain iodine will raise the PBI level to extremely high levels and that this effect can be demonstrated as long as three months after the

treatment is discontinued. It is now generally accepted that the PBI is a measure of the circulating Thyroxin and Triiodothyronine¹⁴.

The adrenal gland has been shown to maintain a comparatively regular diurnal variation^{15, 16, 17, 18} but this type of fluctuation in the thyroid has not been demonstrated as yet. The interrelationship of the adrenal gland with the thyroid is supposed to be mediated through the pituitary gland and not by direct action of the adrenocortical hormones on the thyroid gland. In a study of human subjects at autopsy *Russfield*¹⁹ has shown that there is a similarity in the pathological findings in the pituitary glands of patients having suffered from Addison's Disease and those having suffered from Thyrotoxicosis. Certain cells which he called Aminophils have been reported²⁰ to be depressed by administration of exogenous Cortisone *in vivo*. This has been reported to be of clinical benefit in several conditions which might conceivably be associated with an increased level of Thyroid Stimulating Hormone (hereafter called TSH)^{1, 21, 22}. The fall in the circulating PBI caused by thyroidectomy is accompanied by hypertrophy and hyperplasia of the anterior pituitary Basophils²³. These works indicate that there might be a similar origin of ACTH and TSH from the Aminophils in the pituitary gland. *Levin*²⁴ has shown that the rate of steroid degradation is decreased in Myxedematous patients and increased in those patients suffering from Hyperthyroidism.

Experiments by *Marine* et al.²⁵ demonstrated an increase in thyroid activity in the presence of partial adrenocortical insufficiency. *Gabrilove*²⁶ showed that TSH administered to normal patients does cause a decrease of the 11-Oxysteroid level in the urine whereas the 17-Ketosteroids are relatively unaffected. *Hoskins*²⁷ reported that the adrenal glands in animals increased in weight when the animals were fed dried thyroid glands for a period of time. *Carrol*²⁸ showed this same effect using Thyroxin. Both authors contend that this increase of weight in the adrenals is due to a hypertrophy of the gland. For this effect the pituitary gland must be intact or an atrophy of the adrenal glands will occur instead of the expected hypertrophy. *Wallach* et al.²⁹ showed that the ascorbic acid content of the adrenal gland decreases to minimal values after four days of Thyroxin administration and is then followed by a progressive increase both in adrenal weight and ascorbic acid content of the gland.

Much work has been done with the thyroid gland after the advent of Radioiodine as a means of study. Regarding Radioactive Iodine release by the thyroid gland it has been shown in rats that Cortisone³⁰ produced a prompt, marked, and reversible reduction in the release rate. *Perry*³¹ showed that rats fed on a diet low in iodine showed a decreased Radioactive Iodine (hereafter called RAI) uptake over the thyroid gland when treated previously with exogenous Cortisone or ACTH. He found that neither ACTH nor Cortisone had any effect on the actual weight of the thyroid gland. *Kuhl* and *Ziff*³² showed a decrease in RAI uptake over the thyroid gland during ACTH or Cortisone therapy. These authors contended that

this probably resulted from a suppression of the TSH production by the pituitary gland or from a primary effect on thyroidal activity. *Patschkiss*³³, working with hypophysectomized rats, showed that exogenous TSH increased the uptake of RAI over the thyroid gland while subsequent administration of Adrenocortical hormones depressed this increase in uptake. *Spurr*³⁴ showed that I.V. ACTH in a dosage of 20 mg/day resulted in a reduction of the RAI uptake to approximately half the control level. I.M. ACTH produced an equivalent response to that of I.V. administration and both resulted in a greater inhibition than that produced by Cortisone. Upon withdrawal of the ACTH and Cortisone rebound was not observed. *Migeon*³⁵ showed that RAI uptake in rats treated with Cortisone and in adrenalectomized rats maintained on Desoxycorticosterone acetate was not significantly changed as compared to controls. Adrenalectomized rats maintained on DCA and treated with Cortisone demonstrated a diminished RAI uptake.

Forsham et al.^{35, 36} using both Cortisone and ACTH demonstrated a fall in the PBI level in human subjects. The Cortisone was given intramuscularly and per os in the subjects for a period varying from several days to three weeks. This type of medication caused a decrease in the PBI level from 12 to 60 % of the control values. ACTH administered intramuscularly in doses of 5 to 25 milligrams caused a decrease of PBI in 66 % of the subjects, a slight increase in 22 % and no change in 11 %. In several of their patients receiving Cortisone the maximum decrease was found during the actual period of therapy; in others the maximum fall occurred after therapy was discontinued. All patients showed an upward trend in the PBI level in the first several weeks post treatment. In those patients receiving intramuscular ACTH there tended to be a return of the PBI level toward or above the control value after discontinuance of the hormone. *Wolfson* et al.³⁸ implied that both RAI uptake and PBI values appear to be reliable indices of thyroid function even during administration of ACTH or Cortisone. *O'Neill* et al.³⁹, working with dogs, found that the PBI level fell from a normal of 2.6 ± 1 % to 1 % after hypophysectomy. Single injections of TSH resulted in detectable increases in the PBI level at the end of four hours with a maximum effect at 24–30 hours. This effect lasted for 2–5 days. The concurrent administration of Cortisone did not alter the magnitude or duration of response to TSH. The exogenous Cortisone did not influence the rate of fall of PBI levels following the maximum elevation caused by a single injection of TSH. The Cortisone was given intramuscularly in varying dosages, all of which showed the same ineffectiveness. *Halmi* and *Barker*⁴⁰ treated rats with 2.5 mg of Cortisone every four hours over a twenty-day period and found histological evidence of hypersecretion of Thyreotropin both in the pituitary gland and the thyroid gland in the absence of a fall in the plasma PBI. *Zingg* and *Perry*⁴¹ found no alterations in the plasma PBI levels following three days of intramuscular administration of Cortisone but found a decrease of approximately 1.1 % in the PBI level following administration of Desoxycorticosterone acetate.



Methods and Materials

All subjects used in this study were selected as being normal individuals as concerns their endocrinological functions. Stress had been attempted to be avoided as much as possible by using no patients in severe pain or any who were receiving any form of physical therapy. None of the subjects had received any of the known thyroidal depressant or stimulant drugs or any exogenous iodine over and above the normal daily intake. Seven of the subjects were patients. The other three subjects were normal individuals. Five of the subjects were male and five female. One female (W.G.) was an Italian and one male (K.N.) was a German, both having recently come to Switzerland. Two males (A.H. and F.B.) and one female (E.H.) were all Americans and not confined to the hospital. The remaining five were Swiss. The subjects ranged in age from 19 to 38. The hospital diagnoses of the patients may be seen in the section dealing with each individual under the heading of "results".

All subjects used for study were tested either before treatment had begun or after treatment was completed in those cases which were hospitalized. Two of the patients (A.M. and R.K.) were at complete bed rest; 5 were at partial bed rest; and the remaining three (A.H., E.H., and F.B.) maintained normal daily activities. All the subjects used maintained normal diets, the only restriction being that they eat no fish during the time of study. Blood for the Protein Bound Iodine analysis was taken in all cases after breakfast and lunch. That sample taken at 1600 was taken before dinner but after a light mid-afternoon snack. At one time during the study all patients received an eight-hour infusion intravenously of 25 units of CIBACTHEN (ACTH from Ciba) in a 5% glucose solution, so timed that one half of the liter would have been infused by 1200. All infusions ran from 0800 to 1600.

The length of study varied in these subjects but in all cases blood for examination was drawn at 0800 and 1600 on the days of study and all patients were subjected to PBI determinations at least one day before the ACTH infusion. This one day is meant to serve as a control day so that we might have a base line upon which to estimate the effect of the infusion. Four subjects were also studied to see if there exists a variation in the PBI level during the day. In these cases blood samples were taken at 0800, 1200, and 1600 on every day of study. In all cases blood was

drawn immediately before the infusion was started and directly at the end of the eight-hour infusorial period. In those cases in which blood was taken at 1200 on the infusion day the patients had first eaten lunch and then the blood sample was taken. The same needle was used in taking blood through which the infusion had been running. A tourniquet was applied approximately 10 cm above the infusion point and a minimum of five cc of blood was discarded to prevent infusorial dilution and then the sample was collected. The same method was used for the 1600 sample on the day of the infusion.

Six of the ten patients were used for urinary control studies also, urines being collected in 24-hour samples in two cases and in eight-hour samples (0000-0800, 0800-1600, and 1600-2400) in the other four.

The determination of the Protein Bound Iodine in the plasma was done by a modification of the Barker Method⁴². In this method extreme care must be taken so as to avoid contamination of the sample or loss of material to accurately measure the amount of Protein Bound Iodine in one cubic centimeter of plasma.

From each patient approximately fifteen cubic centimeters of blood was taken and mixed with dry sodium oxalate to prevent clotting. This blood was then centrifuged at 3000-3500 r.p.m. for 15 minutes in an AHT Centrifuge. The supernatant plasma was then drawn off to be used in the determination. Triplicate aliquots of 1 cc of plasma are then pipetted into 15×125 mm Pyrex test tubes and diluted with 6.0 cc of distilled water and 1.0 cc of a 10% zinc sulfate solution (prepared by dissolving 100 grams of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ in a liter of distilled water). The contents of each tube is then thoroughly mixed with glass stirring rods and 1.0 cc of a 0.5 N sodium hydroxide solution is added and the contents again thoroughly mixed. Any material adhering to the glass stirring rod must be removed by rubbing along the inside of the tube to prevent loss of material. The tubes are then centrifuged for 10 minutes at 3000 r.p.m. The supernatant is then discarded and 6.0 cc distilled water is added to each tube and the precipitate resuspended by means of the glass rod. Centrifugation is then carried through in the same manner repeating this entire washing process three times with each tube.

After the last supernatant is discarded, 1.0 cc of 4.0 N sodium carbonate is added to each tube and the sludge thoroughly mixed again, being extremely careful not to carry away any of the sediment. The tubes are then placed in a SAUTER Oven set at 85-95° C and left there for 12-18 hours to drive off any water present. After this process is completed and the precipitate thoroughly dried the tubes are placed in a muffle oven (Borel) set at $600 \pm 25^\circ \text{C}$ and left there for two and a half hours. This process is called "ASHING". The tubes are then left to cool in the oven and later at room temperature.

Two cc of 2 N hydrochloric acid is added carefully to each tube to prevent excessive foaming. A glass rod is then used to mix any unreacted ash with the acid and 2.0 cc of 7 N sulfuric acid and 3.0 cc of distilled

water are added to each tube. The contents are then filtered through ordinary filter paper to remove any insoluble material.

For each sample two specially prepared colorimeter tubes are selected, one of which contains 1.0 cc of distilled water containing 0.04 micrograms of iodine as sodium iodide and the other containing 1.0 cc of distilled water. To each of these tubes three cc of the sample is added and thoroughly mixed. A "Blank" is also prepared by placing 4.0 cc of distilled water in a colorimeter tube. To each tube, including the blank, we add 0.5 cc of 0.1 N sodium arsenite solution which is prepared by dissolving 4.95 grams of As_2O_3 in 25 cc of 4% (1.0 N) sodium hydroxide, slightly acidified with sulfuric acid (as tested with litmus paper) and made up to 1 liter with distilled water. The contents in the colorimeter tubes are now thoroughly mixed by shaking.

The solutions are then placed in a Sauter Oven set at $39 \pm 1^\circ \text{C}$ and left there for ten minutes. A 0.02 N solution of ceric ammonium sulfate, having been made by dissolving 12.65 grams of the salt in 500 cc of water plus 230 cc of 7 N sulfuric acid and then brought up to one liter with water, is also placed in the oven.

"Since the actual determination of iodide is accomplished by measuring the iodide catalysis of the rate of decolorization of the yellow ceric ammonium sulfate by arsenic acid, the times at which the colorimetric readings are made must be definitely set up."⁴²

One cc of the ceric ammonium sulfate is added to each tube and well shaken and the colorimetric reading taken to determine that all tubes in the series are standardized. Approximately 30 seconds should be allowed to elapse before adding the ceric ammonium sulfate to the next tube to allow sufficient time for proper reading at the end. In this laboratory readings are taken at the end of twelve minutes for each tube.

Once the readings have been recorded in each of the tubes the results are then plotted on a semilogarithmic graph. The abscissa of this graph is plotted as time in minutes and the ordinate as colorimetric readings. (It is the ordinate which is arranged in logarithmic progression.) A straight line is drawn between that point of the original reading at 0 minutes in each tube and that point determined by the reading in the tube containing plasma plus the added iodine. Along this line points are made corresponding to that reading in the Blank and that reading in the tube without the added iodine, e.g.

Reading at time 0	325
Reading at 12 min. of plasma + iodine	160
Reading at 12 min. of Blank	300
Reading at 12 min. of plasma + water	240

Determinations:

$$1. \quad 0.04 \times \frac{1.35}{10.65} = 0.00508$$

$$2. \quad 0.04 \times \frac{5.1}{6.9} = 0.02910$$

$$\begin{array}{r} 2. - 1. \quad 0.02910 \\ - 0.00508 \\ \hline 0.02402 \end{array}$$

$$0.02402 \times 233^* = 5.55$$

$$5.55 \times (\text{Standard}^{**} \div 5.8^{***}) = \text{Value in } \gamma\% \text{ of PBI}$$

A summary chemical reaction may be described as follows:



Ce^{++++} is a yellow color

Ce^{+++} is colorless

The colorimetric readings were taken with a Klett Summerson Photo-electric colorimeter using a blue filter. This filter allows only one wave length of light (that at $450 \text{ m}\mu$) to pass and the colorimeter measures the intensity of this wave length of light and so gives us readings of the amount of cerium which was decolorized.

The determinations of the 17-Ketosteroids in the urine (43) were done in the following manner: Urine 17-Ketosteroids are first treated in a water bath at 80°C for 45 minutes with concentrated HCl to hydrolyze water-soluble conjugates. Purified diethyl ether (anhydrous) is used for extraction and the extracts are pooled and treated with 10% NaOH to which 10 grams per 100 cc of $\text{Na}_2\text{S}_2\text{O}_4$ has been added. This is to remove phenolic and acidic impurities and the extracts are then washed two times with distilled water. The neutral residue is dried and then quantitatively determined by the "Callow Zimmerman" reaction. This reaction consists of the formation of a colored complex produced by compounds containing the reactive 17-Keto group with m-dinitrobenzene in an alkaline-alcoholic medium (specifically KOH) which absorbs maximally at $520 \text{ m}\mu$. In this laboratory it is compared to the color developed by a standard of dehydroepiandrosterone.

* Value 233 is taken as a constant to bring the result to the total amount found in 100 cc of plasma. Since 3.0 cc plasma was taken and diluted with 2.0 cc NaOH, 2.0 cc HCl, and 3.0 cc water one must divide by 3 and then multiply by 100 giving 233.

** A standard is made before each series to compare with the normal values as standardized previously for the area and to standardize the iodine solution used in the determination.

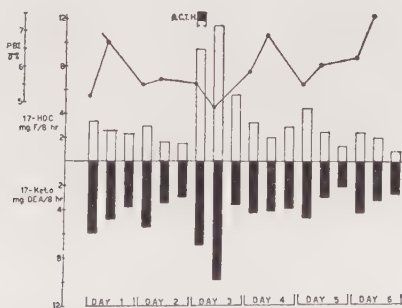
*** The value 5.8 was found to be the value of the PBI in a sample of pooled plasma from 250 soldiers in Switzerland. This is being presently used here as the mean average of PBI.

The chemical determination of 17-Hydroxycorticoids was carried out by the method of *Forsham* and co-workers⁴⁴ in which the butanol extracted hydroxycorticoids were analyzed by the Porter-Silber method. Other interfering substances at 410 m μ were eliminated with the correction as determined by *Allen*⁴⁵.

Two of the subjects tested here were afterward restudied in the same manner as previously with the one exception being that the infusion which they received was a liter of 5% glucose in water without any added ACTH. These two studies were done as controls to determine whether the infusion of glucose alone had any effect on the levels and diurnal variations of PBI. The methods used were exactly the same as described previously.

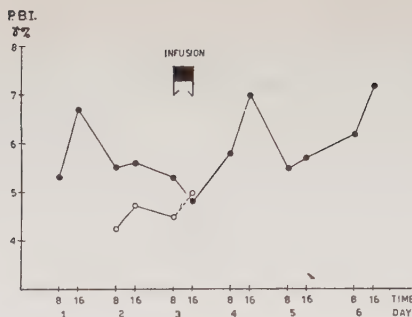
Results

Case # 1, A.H., male, age 27, was tested for 6 days to determine if a diurnal fluctuation in PBI values could be perceived in a normal subject. He maintained normal daily activities, being a medical student and having volunteered for the test. Blood samples were drawn at the hospital at the proper times. Graph # 1 shows a decided fluctuation of PBI levels every day, a lower level being found at 0800 than at 1600. This fluctuation was seen every day of the study with the only exception being during the day of the ACTH infusion at which time the 1600 value was lower than that at 0800. It can also be seen from this graph that the diurnal fluctuation appears to follow a set rhythm, varying equivalently every other day. This subject was also studied as to adrenocortical function by measurement of urinary excretion of corticoids. The results here are of a normal individual.



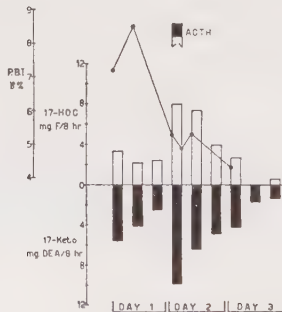
Graph # 1. Urine collections at 0800, 1600, and 2400 daily with blood samples for PBI at 0800 and 1600 daily. Infusion of 1 liter of 5% Glucose solution with 25 units ACTH from 0800 to 1600 on third day.

A control study was done on the same individual (A.H.) 6 months later. This was a two-day study with the only difference being that the infusion of 5% Glucose contained no added ACTH. From this curve (Graph 1A) it can be seen that the glucose infusion itself had no effect on the normal diurnal variation of PBI.



Graph 1A. Comparison of original study (solid circles) with that 6 months later (open circles). The infusion on the latter date was merely 5% Glucose in water while that on the former date was with added ACTH (25 units).

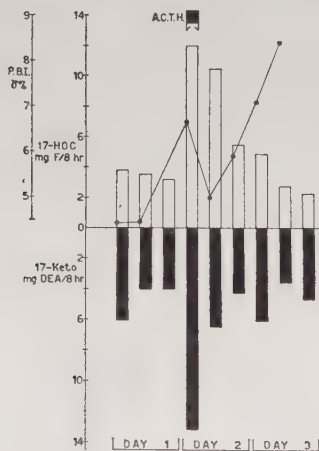
Case # 2, E.H., female, age 24, a normal subject, was studied for three days with the ACTH infusion being given on the second day. The same type of diurnal variation was observed as in the first case. Fortunately, a blood sample was obtained in this case at 1200 on the day of the infusion to determine when the maximum depression of the PBI would occur. Graph # 2 demonstrates that if this blood sample had not been taken we might have been deceived into believing that the ACTH had no effect on the level of the PBI. Urinary steroid studies were completely normal.



Graph # 2. Urine collections at 0800, 1600, and 2400 daily for 3 days with blood samples for PBI at 0800 and 1600. On the day of the ACTH infusion (2) a blood sample was taken at 1200 also.

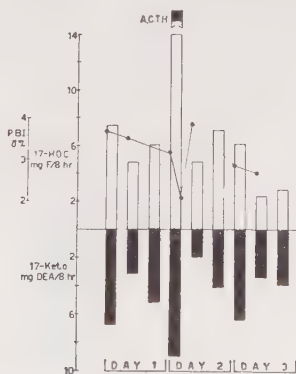
Case # 3, F.B., male, age 26, another medical student, was tested for 3 days. This subject showed no difference in the PBI levels at 0800 and 1600 on the control day. During the ACTH infusion on the second day a decided depression did occur at noon. The PBI levels on the day following the infusion in this case seem to be out of proportion to the

original levels and may be interpreted as being a Rebound type of phenomenon. Urinary steroid studies gave normal results.



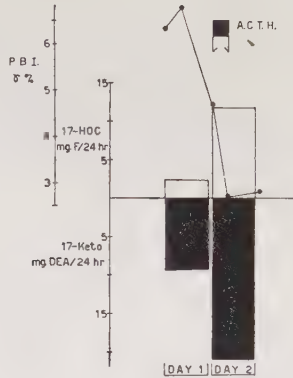
Graph #3. Urine collections at 0800, 1600, and 2400 daily for 3 days with blood samples for PBI at 0800 and 1600.

Case # 4, A.M., male, age 26, a hospital patient with a diagnosis of Discus Hernia L4-5, was studied for 3 days before treatment was begun. Although this patient demonstrated a decided fall on the day of the infusion in the PBI level the results on the day previous to and immediately after the ACTH infusion showed opposite findings to those that have already been obtained in the other patients. On both days the PBI levels of this patient were higher at 0800 than at 1600.



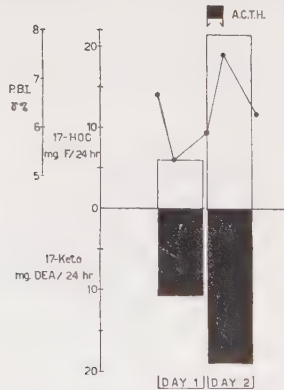
Graph #4. Urine collections at 0800, 1600, and 2400 daily for 3 days with blood samples for PBI at 0800 and 1600. On the day of the ACTH infusion (2) a blood sample was taken at 1200 also.

Case # 5, M.K., female, age 37, a hospital patient with a diagnosis of Discus Hernia L4-5, was studied for 3 days before treatment was begun. The patient showed a diurnal variation of PBI on the day preceding the infusion imitating that seen in three of the previous subjects. As can be seen from Graph # 5 a decided depression of PBI occurred during the ACTH infusion. Urinary steroid studies during the testing showed normal values.



Graph # 5. Urine collections every 24 hours at 0800 for 2 days with blood drawn at 0800 and 1600 on both days and one sample drawn at 0800 on the third day.

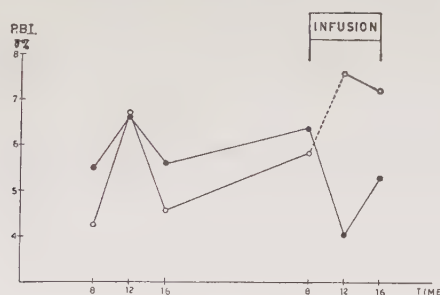
Case # 6, A. H., female, age 44, with a hospital diagnosis of Vegetative Dystonia but with normal endocrine functions, was studied for 3 days and showed a diurnal variation in PBI with a higher value at 0800 than



Graph # 6. Urine collections every 24 hours at 0800 for 2 days with blood drawn at 0800 and 1600 on both days and one sample drawn at 0800 on the third day.

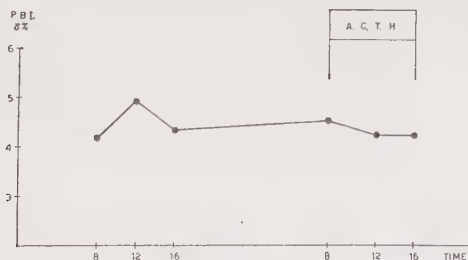
at 1600. During the infusion of ACTH the patient showed a significant, distinctive rise in the PBI value with a fall the following day equivalent to that of the first day. Graph # 6 shows these results which were opposite to those already observed.

Case # 7, E. A., male, age 19, a patient in the hospital with a diagnosis of Spontaneous Pneumothorax, was tested for two days and showed typical results of diurnal fluctuation of PBI with a higher value at 1600 than at 0800. Graph # 7 shows both the original study and a control study done one week later during which the infusion contained no ACTH. In the original study a decided depression in the PBI value may be seen during the ACTH infusion. The control study shows that the glucose infusion did not effect the diurnal variation of PBI.



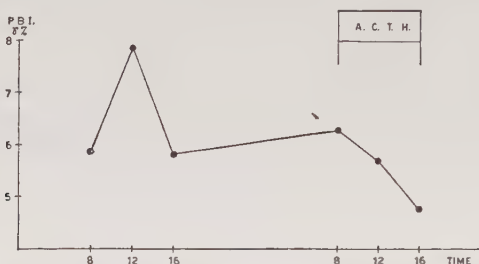
Graph # 7. PBI studies done with an infusion of 5% Glucose in water plus 25 units ACTH (solid circles) as compared to one done 1 week later during which the infusion contained no added ACTH (open circles).

Case # 8, K. N., male, age 35, hospitalized with an admission diagnosis of Bronchitis but with no pathological findings, was studied for two days. He showed results as described above as being typical even though the changes in the PBI levels were of a minimal extent.



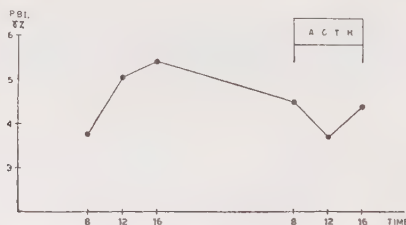
Graph # 8. PBI studies at 0800, 1200, and 1600 for 2 consecutive days with an infusion of 5% Glucose in water with 25 units of ACTH on the second day.

Case # 9, W.G., female, age 20, treated for tonsillitis and tested after all symptoms disappeared, was studied for 2 days. As can be seen on Graph # 9, a marked change of the PBI level occurred on the control day and the levels on the ACTH day were typical in that a depression was observed.



Graph # 9. PBI studies at 0800, 1200, and 1600 for 2 consecutive days with an infusion of 5% Glucose in water with 25 units of ACTH on the second day.

Case # 10, R.K., female, age 36, treated for attempted suicide with sleeping tablets, was tested for two days immediately prior to release from the hospital. She showed a continuous rise in the PBI level during the control day and a depression of the PBI value at noon on the day of the infusion. As can be seen from Graph # 10, a rise occurred on the ACTH day of the 1600 value approaching that at 0800.

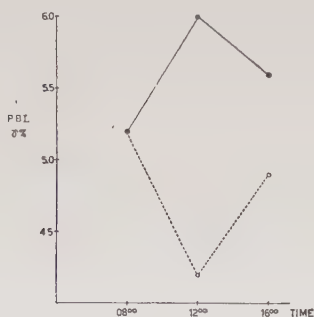


Graph # 10. PBI studies at 0800, 1200, and 1600 for 2 consecutive days with an infusion of 5% Glucose in water with 25 units of ACTH on the second day.

On the basis of previous studies it was decided that three samples of blood be taken on each day from the last four patients instead of the usual two samples. In three of these four cases it can be seen from the graphs that a higher value was obtained at 1200 on the control day than at 0800 or 1600. In all four cases the 1200 value was depressed on the day of the ACTH infusion.

Steroid studies on six of the patients showed normal fluctuations in adrenal function with and without stimulation by exogenous ACTH. These graphs, when taken into consideration to the PBI studies, show a reciprocal relationship as concerns the level of their values.

Graph # 11 shows an average of the absolute values of PBI found in each patient at 0800, 1200, and 1600 both on the control day and the ACTH infusion day. This graph is very illustrative of the effect of exogenous ACTH on the circulating PBI. The depression of the values at 1200 amounts to 1.8 $\gamma\%$ and that at 1600 to 0.7 $\gamma\%$.



Graph # 11. Solid line shows average of all cases of PBI values on control day. Broken line shows average of all cases of PBI values on day of ACTH infusion.

Conclusions

Based on the results found in this study it appears that a reliable percentage of individuals show a variation in PBI values in plasma during the day. This variation, by no means constant from individual to individual, was observed in 8 out of 10 of the cases. *Tingley*¹¹, studying normal individuals for fluctuations in PBI level during three different times each day, found no significant variations. In this study the maximum levels in PBI were higher at 1600 than at 0800 in 8 out of the 10 studied. In the four patients in which a sample of blood was taken at 1200 on the control days 75% showed the highest PBI level to be at that hour. One case (A.H.) was studied for six consecutive days and showed a rise in the PBI level each day except on that day on which he received the ACTH infusion. It is interesting in observing the graph of this individual that one can see a certain relationship between the PBI levels of alternate days. Upon restudying this individual six months later, a similar type of variation may be seen. This would lead one to assume that a variation of PBI levels, though different from individual to individual, tends to remain relatively constant in one individual.

Based on previous studies of adrenal - pituitary - thyroid interrelationships and upon the depressant effect of ACTH and Cortisone upon the PBI level it would seem that the adrenal and the thyroid glands maintain a reciprocal relationship with each other. The known diurnal variation of adrenal function, with maximal activity between 0800 and 1000 gradually diminishing to a minimum between 2200 and 2400^{16, 17, 18}, can be contrasted to that variation shown here in the PBI level which appears to be highest in the afternoon. The six patients who were studied for adrenal variation showed a reciprocal relationship between the PBI level and the steroid output in the urine. The adrenal variation is unaffected by light, food intake, sleep, or exercise and it has been reported that thyroid function is relatively constant also concerning external influences.

The idea of reciprocal interrelationship between the thyroid and the adrenal appears to be borne out by the difference in maximal functioning at different times of the day in each of these glands. Whether this relationship is the result of direct action upon each of these glands by the product of the other, or, whether these glands are solely dependent upon pituitary control and that these effects are only of secondary nature has yet to be determined.

As has been described previously²³, certain cells of the anterior pituitary gland seem to be the precursors of both ACTH and TSH. Upon this presumption certain conclusions, may be drawn; namely that it is neither the adrenals nor the thyroid which vary diurnally but the pituitary and that this variation shows itself by its effect on the other endocrine glands. To support this viewpoint it has been conclusively shown that ACTH does depress the circulating PBI and that this depression, in most cases, is one of rather brief duration. It is known that thyroid function, as studied with RAI, is depressed by ACTH and Cortisone. The fact that Cortisone will depress the thyroid function as well as ACTH seems to detract from the theory that the governing body in this interrelationship is the pituitary.

If one were to assume that the preceding theory, namely that the thyroid-adrenal interrelationship is dependent upon pituitary control, the depression caused by exogenous Cortisone on the thyroid could not be readily explained. If, however, we are to assume that the relationship exists between the thyroid and adrenal glands independent of pituitary control the effect of both Cortisone and ACTH is readily explainable.

Whichever mechanism controls this reciprocal interrelationship between the thyroid and the adrenal glands is as yet unknown and much work must be done in this field to determine the site of action of these hormones. Many other possibilities offer themselves to explain these phenomena, all of which propose more profound explanations rather than the simple action of a hormone upon a gland. Since most of the work concerning the thyroid - pituitary - adrenal interrelationship has been done on laboratory animals, certain theories may be drawn, but to apply these theories to man is a fallacy in itself and the only solution to this problem lies in man himself. Possibilities arise very infrequently to study the endocrine glands in humans which have had one or more glands removed and the use of these individuals for study may also be invalidated due to the upset equilibrium of the organism. However these "gland-ectomized" individuals offer the only solution feasible to the problem of interrelationship of the adrenal with the thyroid and the role of the pituitary gland in this relationship in human beings.

Summary

Ten individuals were studied to determine whether there exists a diurnal fluctuation of PBI and whether the administration of ACTH by infusion will depress the levels of PBI in the blood. Both these problems have been answered to some extent in that ACTH did depress the PBI in 9 out of 10 of the cases and that a diurnal variation exists in PBI levels which appears to be inconstant from individual to individual but relatively constant in one individual.

An attempt has been made to clarify the adrenal-pituitary-thyroid axis to explain the reciprocal relationship between the adrenal and thyroid glands but insufficient data are available to draw any definite conclusions.

Bibliography

1. *Meckestroth, C. V.; Rappaport, R. L.; Curtis, G. M. and Simcox, J.*: The Laboratory Diagnosis of Extrathyroidal Hypermetabolism. *J. Clin. End.* 12: 1373 (1952).
2. *Blackburn, C. M. and Power, M. H.*: Diagnostic Accuracy of Serum PBI Determinations in Thyroid Disease. *J. Clin. End.* 15: 1379 (1955).
3. Editorial: *Annals of Int. Med.* 44: 207 (1956).
4. *Perkin, H. J. and Lahey, D. B.*: Level of Iodine in Blood. *Archives of Int. Med.* 65: 882 (1940).
5. *Perlmuter, M. and Riggs, D. S.*: Thyroidal Collection of Radioactive Iodide and Serum Protein Bound Iodine Concentration in Senescence, in Hypothyroidism, and in Hypopituitarism. *J. Clin. End.* 9: 430 (1949).
6. *Perkin, H. J.; Brown, B. R. and Lang, J.*: Blood Iodine Content of Normal and Thyrotoxic Individuals: Iodine Tolerance Test. *Can. Med. Ass. J.* 31: 365 (1934).
7. *Engstrom, G. W.*: Effect of Stress and Cortisone on the Circulating Thyroid Hormone. *J. Lab. and Clin. Med.* 44: 793 (1954).
8. *Gottschalk, C. W. and Riggs, D. S.*: PBI in Serum of Soldiers and of Eskimos in the Arctic. *J. Clin. End.* 12: 235 (1952).
9. *Danowsky, T. S.; Hedenburg, S. and Greenman, J. H.*: Constancy of Serum Precipitable or Protein Bound Iodine in Healthy Adults. *J. Clin. End.* 9: 768 (1949).
10. *Heinmann, M.; Johnson, C. E. and Man, E. B.*: Serum Precipitable Iodine Concentrations during Pregnancy. *J. Clin. Investigation* 27: 91 (1948).
11. *Tingley, J. O.; Morris, A. W. and Richardson Hill, S.*: Studies on Diurnal Variation and Response to Emotional Stress on the Thyroid Gland. *Clinical Research* 6: 134 (1958).
12. *Basset, A. M.; Coons, A. H. and Salter, W. T.*: Protein Bound Iodine in Blood; Naturally Occurring Iodine Fractions and their Chemical Behavior. *Am. J. Med. Sc.* 202: 516 (1941).
13. *McClendon, J. F. and Foster, W. C.*: Protein Bound Iodine in Erythrocytes and Plasma. *J. Biol. Chem.* 154: 619 (1944).
14. *Goodman, L. S. and Gillman, A.*: Pharmacological Basis of Therapeutics 2: 1532 (1956).
15. *Pincus, G.*: A Diurnal Rhythm in the Urinary Excretion of Ketosteroids by Young Men. *J. Clin. End.* 3: 195 (1943).

16. *Tyler, F. M.; Migeon, C.; Thorentin, A. A. and Samuels, L. T.*: The Diurnal Variation of 17 Hydroxycorticoid Levels in Plasma. *J. Clin. End. and Met.* **14**: 774 (1954).
17. *Sandberg, A. A.; Nelson, D. H.; Glenn, E. M.; Tyler, F. M. and Samuels, L. T.*: 17 Hydroxycorticoids and 17 Ketosteroids in Urine of Human Subjects. *J. Clin. Invest.* **32**: 818 (1953).
18. *Laidlaw, J. C.; Jenkins, D.; Reddy, W. J. and Jacobson, T.*: The Diurnal Variation in Adrenocortical Secretion. *J. Clin. Invest.* **33**: 950 (1954).
19. *Russfield, A. B.*: Histology of the Human Hypophysis in Thyroid Disease – Hypothyroidism, Hyperthyroidism, and Cancer. *J. Clin. End.* **15**: 1391 (1955).
20. *Russfield, A. B.*: The Endocrine Glands after Bilateral Adrenalectomy Compared with those in Spontaneous Adrenal Insufficiency. *Cancer* **8**: 523 (1955).
21. *Kinsell, L. W.; Partridge, J. W. and Foreman, N.*: The Use of ACTH in the Treatment and in the Differential Diagnosis of Malignant Exophthalmos. *Annals of Int. Med.* **38**: 913 (1953).
22. *Werner, S. C.*: ACTH and Cortisone Therapy of Acute Nonsuppurative (Subacute) Thyroiditis. *J. Clin. End. and Met.* **13**: 1332 (1953).
23. *Goldberg, R. C. and Chaikoff, I. L.*: Failure of Dinitrophenol Induced Fall in Plasma Protein Bound Iodine to Stimulate Augmented TSH Production. *Endocrinology* **49**: 613 (1951).
24. *Levin, M. E. and Daughaday, W. H.*: The Influence of the Thyroid on Adrenocortical Function. *J. Clin. End.* **15**: 1499 (1955).
25. *Marine, D. and Baumann, E. J.*: Influence of Glands with Internal Secretions on Respiratory Exchanges VI. Further Observations on the Effect of Suprarenal Insufficiency (by Removal) in Rabbits. *J. Met. Research* **2**: 1 (1922).
26. *Gabrilove, J. L. and Soffer, L. J.*: Effect of Thyrotropin on Adrenocortical Function. *J. Clin. End.* **15**: 585 (1955).
27. *Hoskins, R. G.*: Thyroid Secretion as a Factor in Adrenal Activity. *J. Am. Med. Ass.* **55**: 1724 (1910).
28. *Carrol, K. K. and Noble, R. L.*: Effects of feeding Rape Oil on some Endocrine Functions of the Rat. *Endocrinology* **51**: 476 (1952).
29. *Wallach, D. P. and Reinche, E. P.*: The Effect of Varying Levels of Thyroidal Stimulation on the Ascorbic Acid Content of the Adrenal Cortex. *Endocrinology* **45**: 75 (1949).
30. *Brown-Grant, K.*: Inhibition of Release of Thyroidal RAI in the Rat by Cortisone. *Endocrinology* **56**: 607 (1955).
31. *Perry, W. F.*: The Action of Cortisone and ACTH on Thyroid Function. *Endocrinology* **49**: 284 (1951).
32. *Kuhl, W. J. and Ziff, M.*: Alteration of Thyroid Function by ACTH and Cortisone. *J. Clin. End.* **12**: 554 (1952).
33. *Patschkiss, K. E.; Epstein, D.; Cantarow, A. and Friedler, G.*: Influence of Adrenal Hormones on Thyroid Function in the Hypophysectomized Rat. *J. Clin. End.* **12**: 939 (1952).
34. *Spurr, A. J.*: I-131 Uptake by Thyroid after TTH, ACTH, and Cortisone. *J. Lab. and Clin. Med.* **40**: 946 (1952).

35. *Migeon, C. J.; Gardner, L. I.; Crigler, J. F., Jr. and Wilkins, L.*: Effect of Cortisone Treatment for 28 Days on RAI Metabolism in Normal Rats and Adrenalectomized Rats maintained with Desoxycorticosterone. *Endocrinology* 51: 117 (1952).
36. *Fredrickson, D. S.; Forsham, P. H. and Thorn, G. W.*: Effect of Massive Cortisone Therapy on Measurements of Thyroid Function. *J. Clin. End.* 12: 541 (1952).
37. *Hill, S. R.; Reiss, R. S.; Forsham, P. H. and Thorn, G. W.*: Effect of ACTH and Cortisone on Thyroid Function: Thyroid-Adrenocortical Interrelationships. *J. Clin. End.* 10: 1375 (1950).
38. *Wolfson, W.*: Corticogenic Hyperthyroidism. *J. Lab. and Clin. Med.* 36: 1005 (1950).
39. *O'Neal, L. W. and Heinbecker, P.*: The Response of PBI of Hypophysectomized Dogs to Injected Thyrotropin. The Influence of Cortisone. *Endocrinology* 53: 60 (1953).
40. *Halimi, N. S. and Barker, S. B.*: Histo-Pharmacological Effects of Cortisone on Rat Pituitary and Thyroid. *Endocrinology* 51: 127 (1952).
41. *Zingg, W. and Perry, W. F.*: The Influence of Adrenal and Gonadal Steroids on the Uptake of I by the Thyroid Gland. *J. Clin. End.* 13: 712 (1953).
42. *Barker, S. B.; Humphrey, J. and Soley, M. H.*: The Clinical Determination of PBI. *J. Clin. Invest.* 30: 55 (1951).
43. *Walser, A.; Hess, W.; Meier, A. L. and Spring, P.*: Adreno-lienaler Shunt beim metastasierenden Mammakarzinom. *Deutsche Med. Wochenschrift* 14: 580 (1958).
44. *Forsham, P. H.; Di Raimondo, V.; Island, D.; Rinfret, A. and Orr, R.*: Ciba Foundation; Colloquia on Endocrinology. Vol. 8: 279 (1955).
45. *Allen, W. M.*: Simple method for Analyzing Complicated Absorption Curves, of Use in Colorimetric Determination of Urinary Steroids. *J. Clin. End. and Met.* 10: 71 (1950).

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